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# ENZYMATIC ACTIVITY AND FINE STRUCTURE OF *SPODOPTERA LITTORALIS* LARVAL MID GUT TREATED WITH AN INSECT GROWTH REGULATOR AND TWO BIOCIDES

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One IGR and two bioinsecticides had bean studied to determine the activity of some carbohydrases in *Spodoptera littoralis* larval homogenate. Amylase activity was significantly increased after treatment with flufenoxuron and *B. thuringiensis* not after treatments with a nuclear polyhydrosis virus SNPV. Invertase activity was not affected in both flufenoxuron and polyhydrosis virus treatments, but significantly decreased after *B. thuringiensis*. Trehalase activity was not affected after treatment with *B. thuringiensis*, but significantly after treatment with flufenoxuron or SNPV.

Treatment of 4th instar larvae *S. littoralis* with both flufenoxuron and *B. thuringiensis* resulted in severe histological changes in the midgut of the surviving late 6th instar larvae. The flufenoxuron caused collapsing and lysis of the columnar epithelial cells with a higher magnitude in case of treatment with *B. thuringiensis*. The most prominent changes induced by flufenoxuron were the vacuolation of the epithelial cells and disruption of the peritrophic membrane.

**Keywords:** *Spodoptera littoralis*, Bioinsecticides, Histological midgut, Biochemical.

Insect pathogens commonly used for pest control not only pathogenic to mammals. Indeed, the pathogen is usually capable of infecting only a limited number of insect species. Insect pathogens are extremely safe comparison to chemical insecticides acts on insect metabolism, ultimately affecting development and growth of the target insect, particularly when such compounds are applied during the sensitive period of insect development. They induce morphological abnormalities as well as death of treated insects. Many IGRs have shown high potentiality against lepidopteraus insect (El-Deeb *et al.*, 1991; Abdalla and Sammour, 1992; Fisk and Wright, 1992; Rao

*et al.*, 1994 and Sokar, 1995). They also affect activity in *S. littoralis* (Ahmed *et al.*, 1990; Auda and Salem, 1997; Farag, 2001 and Abdel-al, 2003). Several authors studied the probit analysis (Finney, 1971) to obtain the $LC_{50}$ values. histopathological effects of *Spodoptera littoralis* (Abo El-Ghar *et al.*, 1994 and Abdel-al, 2003).

The present study aims to evaluate three tested compounds for controlling *S. littoralis* larvae and to explore of the mode of action of the treatment by studying their effects on the specific activity of certain digestive enzymes and histopathological studies on the midgut.

Egg masses of the cotton leafworm, *Spodoptera littoralis* were obtained from the Research Division of the Cotton Leafworm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands. They were fed on castor bean leaves, *Ricinus communis*, at 27±2°C and 65 ± 5% RH and examined daily. As larvae reached the 4th instar, they were used in the experiments described below.

The treatments were: untreated control; flufenoxuron 10 % obtain from sumitomo chemical Co., Ltd., Spodoptera nuclear polyhydrosis virus SNPV; the production, preparation, formulation, testing and application of a microbial pesticide were carried out according to the methods and techniques adopted by Mckinley (1985) at the rate of $7\times10^5$/ feddan. Dipel 2X ($32\times 10^3$ IU/ mg.), (*Bacillus thuringensis* var. *Kurstaki*) valent Berlner chemical Co.

Susceptibility of *S. littoralis* larvae to the IGR was carried out by using the leaf-dipping technique (Abo El-Ghar *et al.*, 1994). Different aqueous concentrations of the flufenoxuron, *B .thuringiensis* and polyhydrosis virus respectively were prepared. Castor bean leaves, *R. communis*, were dipped in each concentration, 4, 2, 1, 0.5 and 0.25 x $10^5$ Bip/ larva) for SNPV and 8, 6, 4, 2 and 1 (IU) for B.T and 6, 4, 2, 1 and 0.5 ppm for flufenoxuron, then left to dry at room temperature and then were offered to the newly moulted 4th instar larvae. Four replicates (20 larvae/each concentration) were used. Larvae that fed on untreated castor bean leaves were used as control. Larvae were allowed to feed for 24 hrs. then, they were provided with fresh untreated castor bean leaves until pupation. The percent mortality of treated larvae were corrected against those of the control by using Abbott's formula (Abbott, 1925). The data were then subjected to probit analysis (Finney, 1971) to obtain the $LC_{50}$ values.

**Biochemical Studies**

**Preparation of samples for biochemical analysis**

Samples were collected according to the method described by Ishaaya *et al.*(1971). The enzyme solutions samples were obtained by homogenizing the 6th instar larvae representing 1 gm of larval body weight in 10 ml distilled water by using chilled glass Teflon grinder. The homogenate was

centrifuged for 10 min. at 5000 rpm and 5°C, the supernatant fraction being used for the enzyme assay.

**Determination of carbohydrases activity**

Amylase, invertase and trehalase activities were determined according to the method reported by Ishaaya and Swirski, (1970) and Ishaaya *et al.*, (1971) using 3,5 dinitrosalicylic acid reagent for determining the free aldehydic groups of glucose formed after starch, sucrose or trehalose digestion. This reaction is based on the reduction of dinitrosalicylic acid by aldehydic groups of glucose units in basic medium. The reduced dinitrosalicylic acid was measured spectrophotometrically at an absorbace of 550 nm.

## Histopathological studies

The tested compounds were selected for a comparative study to clarify their effects on the histology of the midgut of late 6th instar larval surviving after treatment of the 4th instar larvae with the $LC_{50}$ values of the tested compounds. The tested tissues were fixed in aqueous Bouin's solution for 24 hrs. The normal paraffin wax embedding procedure was followed. The sections were cut at 6μ thick and stained with heamatoxylin and eosin for microscopic examination. Control sections of non-treated larvae were also carried out.

# RESULTS AND DISCUSSION

## Toxicological and Biochemical Effects

$LC_{50}$ values of the tested compounds against the newly moulted 4th instar larvae recording 0.303 ppm, 6.031 IU gm / L and 1.3 X $10^5$ PIB / ml for flufenoxuron, *B. thuringiensis* and polyhydrosis virus respectively (Table 1).

**TABLE (1). Susceptibility of *Spodoptera littoralis* (Boisd.) 4th instar larvae to SNPV, flufenoxuron and *B.thuringiensis*.**

| Tested compounds | $LC_{50}$ (ppm) | 95% Fiducial limits | | Slope ± S.E | $X^2$ |
|---|---|---|---|---|---|
| | | Lower | Upper | | |
| Nuclear polyhedrosis virus (SNPV) | 1.3 X $10^5$ BIP / Larvae | 01.5 | 2.9 | 1.3 ± 0.07 | 0.841 |
| Flufenoxuron | 0.303 | 0.176 | 0.534 | 1.22 ± 0.270 | 0.162 |
| *B.thuringiensis* | 6.03 (IU)* | 5.1 | 13.5 | 2.02 ± 0.260 | 11.98 |

* International unit (the amount of enzyme which under defined assay conditions will catalyze the conversion of one micromole of substrate per minute)

Amylase activity was increased significantly in case of flufenoxuron and *B. thuringiensis* treatments, while insignificantly in case of neuclear polyhydrosis virus treatment as compared to control ( Table 2). On the other

hand, invertase activity was decreased significantly in both flufenoxuron and nuclear polyhydrosis virus treatments, and insignificantly in case of *B. thuringiensis* treatment. Lastly, trehalase activity was decreased insignificantly in the case of *B. thuringiensis*, and significantly after treating with the other two tested compounds as compared to control.

**TABLE (2). Effect of $LC_{50}$ of flufenoxuron, Nuclear Polyhedrosis Virus (SNPV) and *B.thuringiensis* on the all body tissue carbohydrases activity of late 6th instar larvae of *S. littoralis*.**

| Tested Compounds | Mean carbohydrases activity (IU/ml) ± S.E | | |
|---|---|---|---|
| | Amylase | Invertase | Trehalase |
| Flufenoxuron | 297.5 ± 0.28*** | 472.4 ± 0.60 ns | 212.7 ± 0.12*** |
| SNPV | 209.8 ± 0.46 ns | 474.2 ± 0.30 ns | 196.9 ± 0.30** |
| *B. thuringiensis* | 330.5 ± 1.55*** | 405.3 ± 0.88** | 303.7 ± 0.40 ns |
| Untreated check | 205.4 ± 1.20 | 478.2 ± 0.60 | 313.3 ± 1.10 |

ns: not significant.

** and *** significant at P< 0.01 and P< 0.001 respectively.

In insects, amylase and invertase, which have been reported to occur in the digestive tract of several insects, are important for digestion and utilization of starch and sucrose, respectively. Decreased activity of invertase in the homogenate of late 6th instar Larvae of *S. littoralis* due to treatment with the chitin synthesis inhibitors, flufenoxuron agree with the data obtained by Auda and Salem (1997) and Abdel-al (2003). Ishaaya *et al.* (1971) demonstrated the inhibitory effect of certain antifeedants (triphenyltin derivatives) on the digestive enzymes, amylase, protease, and invertase of *S. littoralis* larvae that were fed on castor bean leaves treated with such chemicals.

Trehalase is generally present in large amounts in the haemolymph of most insects and it has an important function as an energy supply in insects and, thus, its activity migut serve as an indicator of energy reserves resulting from availability of carbohydrate nutrient. The trehalose- trehalase system is activated for the production of glucose needed, probably for chitin build-up in the newly synthesized cuticle (Clarke and Jewess, 1990). The partial inhibition of trehalase enzyme in the present work is similar to Abdel-al (2003) in the haemolymph of the same insect species treated with flufenoxuron and might affect this system. The rapid decrease of glucose concentration at the end of the last larval instar *S. littoralis* larvae was probably caused by high metabolic activity of the epidermis, which is known as a tissue with low trehalase, so it is unable to utilize trehalose. In explanation of the reduction which occurred in larval digestive enzymes activities, Ishaaya *et al.*, (1971) suggested that *in vivo* the inhibitory effects of phenyltin compounds on the digestive enzymes may result from its binding to inactive (zymogens) or active digestive enzymes.

**Histopathological Effects**

Light microscopic examination shows that the histological structure of the midgut of 6th instar *S. littoralis* larvae is simple (Fig. 1). It is composed of two layers; the longitudinal muscle fibers is the outer layer and the circular muscle in the inner layer. The circular muscle fibers constitute the principal layer of the musculosa and are very close to the basement membrane of the epithelial cells. The longitudinal muscle fibers lying external to the circular ones are few and widely spaced. Next to the musculosa inwards is a basement membrane, which is followed by an epithelial layer of cells lining the midgut cavity. Within the lumen there is a thin peritrophic membranes, surrounding the food mass. The epithelium consists of a single layer of several types of cells, three of them are principal; columnar, goblet and regenerative (interstitial) cells. The columnar cells are cylindrical and every one contains a large coarsely nucleus occupying a middle position within the cell. These cells are striated, having brush-like border (microvilli). The goblet cells are somewhat calyx-shaped and are seen in great numbers between the columnar cells; each has in its mesal part a large ampulla opening by a narrow neck through a small aperture on the inner surface. The nucleus of this cell type lies at the basal end of the cell. The regenerative cells are small and rest on the basement membrane between the bases of the other cells Each cell is round or elongated and contains a large nucleus surrounded by a small amount of strongly basophilic cytoplasm.

Light microscopic examination shows that treatment of *S. littoralis* 4th instar larvae with the $LC_{50}$ of flufenoxuron caused exfoliation of the midgut epithelium from the underlying circular muscle fibers, leaving a large vacuole or space (Fig.2). Vacuolization of the midgut epithelium and disruption of both the peritrophic membranes and the striated border were evident the same observation was found after treating the larvae with polyhydrosis virus (Fig.3). The nuclei of the columnar cells of larvae treated with flufenoxuron were pycnotic. Some of the degenerated columnar cells were fused with the disrupted peritrophic membrane. The lumen of the gut was collapsed, and globular bodies and cytoplasmic fragments were seen pinching off or exuding from the tip of some epithelial cells within the lumen vicinal to the deteriorated peritrophic membrane. The general appearance of the cytoplasm turned reticular and the regenerative cells at the base of the epithelium disintegrated. Such disintegrated regenerative cells lost their integrity and identity as a compact basophilic mass in between the bases of columnar epithelium. The muscularis lost their compact appearance and the circular muscle layer was vacuolated.

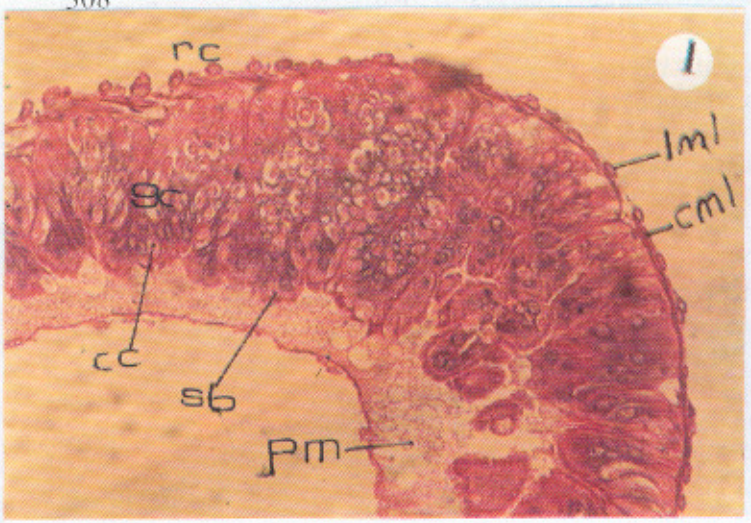


**Fig (1). Photomicrograph of L.S. in the mid gut of normal late 6th instar larvae of *S. littoralis* (X400).**

cc: Columnar cell.
cml: Circular muscle layer.
gc: goblet cell.
Iml: longitudinal muscle layer.
Pm: Peritrophic membrane.
rc: Regenerative cell.
Sb: Striated border
v: vacuole

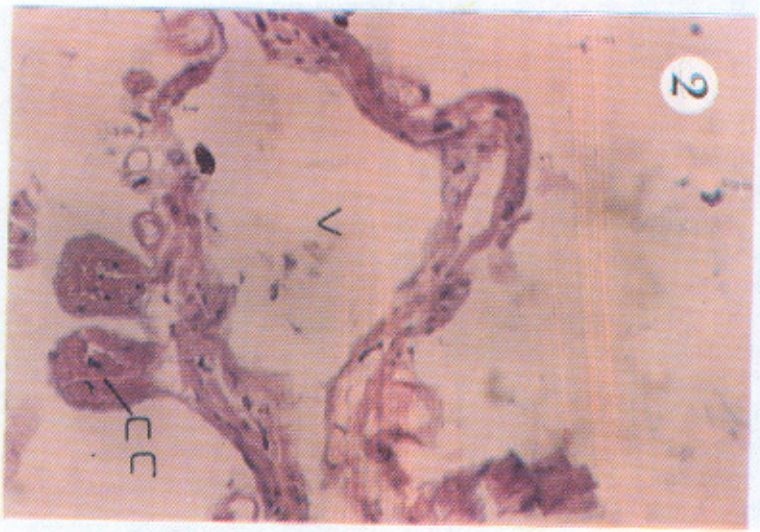


**Fig (2). Photomicrograph of L.S. in the mid gut of late 6th larval instar of *S. littoralis* treated with $LC_{50}$ of flufenoxuron (0.3 ppm) as 4th instar larvae (X400)**

On the other hand, treatment with *B. thuringiensis* (Fig 4) caused that the nuclei of the columnar cells were pycnotic. Some of the degenerated

columnar cells were fused with the disrupted peritrophic membrane. The general appearance of the cytoplasm was reticular and coagulated; and the regenerative cells at the base of the epithelium disintegrated. Disintegrated regenerative cells lost their integrity and identity as a compact basophilic mass in between the bases of columnar epithelium. The effect of the polyhydrosis virus (Fig 3) show that the lumen of the gut was collapsed, globular bodies, and cytoplasmic fragments were observed pinching off from the tip of some of the epithelial cells within the lumen vicinal to the deteriorated peritrophic membrane. The muscular is lost their compact appearance. Vacuolization and exfoliation of the columnar cells and loss of many circular muscles in the late 6th late instars. The lumen of the midgut epithelium was highly shrunken. Few cytoplasmic fragments were seen pinching off from the tip of the epithelial cells underneath the peritrophic membrane, and the regenerative cells lost their integrity.

Many of the histological alterations reported in the present study for the midgut of *S. littoralis* late 6th instar larvae treated with flufenoxuron in the 4th instars agree with those reported by other authors. Abo El-Ghar *et al.* (1994) reported that 0.01 ppm of diflubenzuron caused vacuolization of the midgut epithelium of *S. littoralis* larvae, in addition to the sloughing off scattered groups of the midgut epithelium into the gut lumen and disappearance of the cell boundaries. Similar observations were also recorded for *Aedes aegypti* larvae (Syafruddin *et al.*, 1990) the cat flea adult, *Ctenocephalidis felis* (Meola, *et al.*, 1996) and *S. littoralis* late 6th instar larvae (Abdel-al, 2003) when all were treated with IGRs.

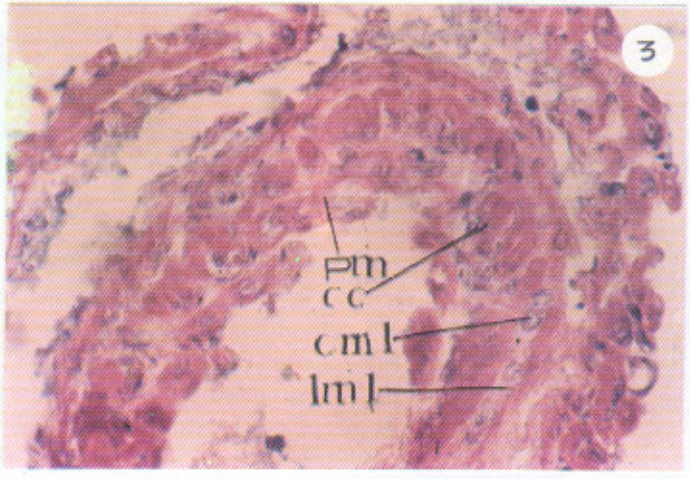


**Fig (3). Photomicrograph of L.S. in the mid gut of late 6th larval instar of *S. littoralis* treated with $LC_{50}$ of NPV as 4th instar larvae (X400).**

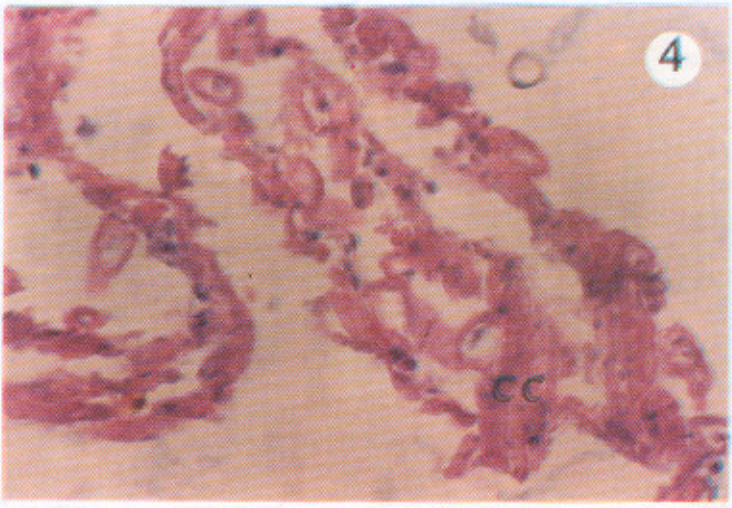


**Fig (4). Photomicrograph of L.S. in the mid gut of late 6th larval instar of *S. littoralis* treated with $LC_{50}$ of B.T.K. as 4th instar larvae (X400).**

In view of the results obtained in the present study, it can be generally pointed out that the general disturbances in carbohydrate metabolism, as expressed by inhibition of trehalase, invertase and amylase activities, may resulted from a chain effect originating primarily from inhibition of chitin synthesis. Alteration in glucose level which may resulted from the inhibition of chitin synthesis could be one of the reasons for reduced activity observed in various carbohydrate hydrolyzing enzymes. On the other hand, either histolgical allteration of the midgut or disturbance of digestive enzymes as the result of treatment with *B. thuringiensis* or polyhydrosis virus revealed that the mode and site of action of the *B. thuringiensis* and the polyhydrosis virus more or less concentrated in the midgut of treated insect.

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# النشاط الأنزيمى والتركيب الدقيق للمعى الأوسط لليرقات دودة ورق القطن المعاملة بمنظم النمو واثنين من المركبات الحيوية

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اشتملت الدراسة على تقدير تأثير بعض المعاملات على نشاط الأنزيمات الهاضمة للكربوهيدرات في دودة ورق القطن وقد وجد أن نشاط إنزيم الأميليز يزيد زيادة معنوية في حالة المعاملة بمركب الفلوفينوكسيورون والباسلليس ثيورينجينسيز ولكن هذه الزيادة كانت غير معنوية في حالة المعاملة بمركب الفيروس النووي المفرد بالمقارنة بغير المعاملة (المقارنة).

من ناحية أخرى أدت المعاملة إلى نقص بدرجة غير معنوية فى نشاط إنزيم الانفرتيز فى حالة مركب الفلوفينوكسيورون والفيروس النووي المفرد ولكن كان النقص معنوي فى حالة المعاملة بمركب والباسلليس ثيورينجينسيز بالمقارنة بغير المعاملة. على العكس فى حالة نشاط إنزيم التربهاليز قد نقص نشاط إنزيم التربهاليز كان النقص غير معنوي فى حالة المعاملة بمركب الباسلليس ثيورينجينسيز ونقصا معنويا فى حالة مركب الفلوفينوكسيورون والفيروس النووي المفرد بالمقارنة بغير المعاملة.

أحدثت كذلك معاملة الطور اليرقي الرابع لدودة ورق القطن بمركب الفلوفينوكسيورون ومركب الباسلليس ثيورينجينسيز تغيرات هستولوجية شديدة فى المعي الأوسط ليرقات الطور السادس المتأخر الناتج من تلك المعاملة. أحدثت كذلك المعاملة بمركب الفلوفينوكسيورون تقلصا وتحللا فى الخلايا العمودية ولكن هذا التأثير كان أكثر فاعلية فى حالة مركب الباسلليس ثيورينجينسيز وكان أكثر التأثيرات وضوحا هو تكوين فجوات كبيرة فى الخلايا الطلائية وتهتك للغشاء البلازمي.